Citation:

Ganji V, Kafai MR. Trends in serum folate, RBC folate, and circulating total homocysteine concentrations in the United States: Analysis of data from National Health and Nutrition Examination Surveys, 1988-1994, 1999-2000, and 2001-2002. *J Nutr.* 2006 Jan; 136 (1): 153-158.

PubMed ID: 16365075

Study Design:

Trend study

Class:

D - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

Utilizing data from the National Health and Nutrition Examination Survey (NHANES) 1988 to 2002, the purpose of this study was to investigate the changes in serum folate, RBC folate and total homocysteine (tHcy) concentrations among residents in the United States.

Inclusion Criteria:

- Individuals included in the NHANES (National Health and Nutrition Examination Surveys)
- United States' civilians residing in the US
- Non-institutionalized
- Greater than or equal to two months of age.

Exclusion Criteria:

- Pregnant women
- Individuals who had fasted less than 10 hours before phlebotomy
- Persons with missing values for serum folate, RBC folate and tHcy concentrations
- Persons less than two months of age.

Description of Study Protocol:

Recruitment

NHANES data used in this study were derived from the databases released for public use by the National Technical Information Service, Springfield, VA.

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Design

Trend study (data derived from NHANES, which was a complex, stratified multistate probability sample survey).

Intervention

Folic acid fortification policy.

Statistical Analysis

- SUDDAN statistical software was used to account for complex survey design. Sample weights, primary sampling units and stratification variables were considered in the analysis so that the differential probabilities of selection and adjustments for non-coverage and non-response bias were taken into account
- SAS was also used to manage and analyze data files
- Due to the asymmetric nature of the data, geometric mean serum folate, RBC folate and tHcy concentrations were determined according to sex and race/ethnicity
- Prevalence (percentage) of at-risk for low serum folate (less than 6.8nmol per L), low RBC folate (less than 362.6nmol per L) and high tHcy concentrations (more than 13umol per L) were determined
- Standard efforts were determined using the Taylor series linearization method
- Bonferroni adjustment was used to determine the at-risk for low serum folate, low RBC folate and high tHcy concentrations after testing the hypothesis with T-test for independent samples
- Sex-, age- and race/ethnicity-adjusted mean serum folate, RBC folate and tHcy concentrations across the three NHANES were done by analysis of covariance (ANCOVA)
- Differences in sex-, age- and race/ethnicity-adjusted mean serum folate, RBC folate and tHcy concentrations across the three NHANES were analyzed with Bonferroni adjustment after testing the hypothesis with ANCOVA.
- Data are presented as means ±SE
- In all other analyses, P=0.05 was considered significant.

Data Collection Summary:

Timing of Measurements

Serum folate, RBC folate and tHcy were measured at three time points:

- NHANES III (1988 to 1994)
- NHANES (1999 to 2000)

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- NHANES (2001 to 2002).

Dependent Variables

- Serum folate levels
- RBC folate levels
- tHcy (circulation total homocysteine) concentrations.

Independent Variables

Folic acid fortification.

Control Variables

- Sex (male and female)
- Age (18 or less, 19 to 30, 31 to 50, 51 to 70, older than 70 years)
- Race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American/Hispanic, others)
- Poverty Income Ratio (PIR) (less than 1.0, 1.0 to less than 2.5, 2.5 to less than 4.0, 4.0 or more).

Description of Actual Data Sample:

- *Initial N:*
  - NHANES III included 39,695 subjects
  - NHANES 1999-2000 included 9,965 subjects
  - NHANES 2001-2002 included 11,039 subjects
- *Attrition (final N):* Data analysis was based on 17,144 measurements for serum folate, 17,213 for RBC folate and 11,415 measurements for circulating tHcy concentrations across three surveys
- *Age:* Two or more months of age
- *Ethnicity:* Reported as
  - Non-Hispanic white (NHW)
  - Non-Hispanic black (NHB)
  - Mexican-American/Hispanic (MA/H)
  - Others
- *Other relevant demographics:* Reports PIR, which is used to define socioeconomic status
  - PIR is the ratio of a family's income to their appropriate threshold income. Income with a PIR value less than 1.0 is considered to be below the poverty level
  - In this study, PIR is categorized as less than 1.0, 1.0 to less than 2.5, 2.5 to less than 4.0, and 4.0 or more.
- *Location:* United States.

Summary of Results:

- Geometric mean serum folate concentrations were 149.6 and 129.8% higher in 1999 to 2000

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and 2001 to 2002, respectively, than in 1988 to 1994 (P<0.0001)
- Across the three surveys, serum and RBC folate concentrations were higher in women than in men, in NHW than in NHB and MA/H, in older persons than in younger persons an in persons with high PIR than those with low PIR. Sex-, age-, and race/ethnicity-adjusted serum folate was significantly lower in 2001 to 2002 than in 1999 to 2000 (10.4%, P<0.0002)
- The prevalence of low serum folate decreased from 18.4% in 1988 to 1994 to 0.8% in 1999 to 2000 and to 0.2% in 2001 to 2002 (P<0.0001)
- RBC folate increased from 391nmol per L in 1988 to 1994 to 618nmol per L in 1999 to 2000, and to 611nmol per L in 2001 to 2002. Consequently, the prevalence of low RBC folate decreased from 45.8% in 1988 to 1994 to 7.3% in 1999 to 2000 and to 7.1% in 2001 to 2002 (P<0.0001)
- Although, RBC folate status improved after folic acid fortification in all race/ethnicity, the prevalence of low RBC folate (approximately 20.5%) continues to be high in non-Hispanic blacks
- Age-, sex-, and race/ethnicity-adjusted tHcy declined from 9.5mmol per L in 1988 to 1994 to 7.6mumol per L in 1999 to 2000 and to 7.9mmol per L in 2001 to 2002.

Author Conclusion:

- Folic acid fortification appears to have contributed to significant improvement in folate status in all segments of the US population. However, this improvement may not be sustained due to decreased serum folate concentrations recently
- This may be due to lower folic acid intakes (associated with a decrease in folic acid added to fortified cereals).

Reviewer Comments:

- *Statistical comparisons between NHANES 1988-1994 and NHANES 1999+ should be interpreted with caution; the authors state that there are methodological differences in measuring tHcy concentrations. These differences were caused by a change in testing methods from testing plasma to testing serum*
- *No funding source was given.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes

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4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	???
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	???
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	Yes
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes

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4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	No
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	No
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A

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7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	N/A
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	N/A
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	No
10.2.	Was the study free from apparent conflict of interest?	Yes

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